

Appendix 6: Detailed Description of the Proposed Overall Risk Assessment Scheme

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A6.1 SPRAYED TREATMENTS

1. Details of the product and its pattern of use

The most important route of exposure of honey bees to plant protection products for spray applications is by direct exposure to field sprays. In some cases, exposure of bees is not possible and there is no need for a detailed assessment of risks, such as in the case of products used during winter when bees are not foraging, pre-emergent herbicides where plants may not be present to forage on, indoor residential uses and uses in glasshouses where bees are not used for pollination. However, in any scenario where, irrespective of the timing of application, the presence of residues in flowers cannot be excluded the potential for bee exposure should be considered.

The attractiveness of the crop to honey bees may be considered as an entry point for this risk assessment. Useful guidance in this respect may be found in the MRL Working Group (EC, 2009) publication which includes additional criteria to consider, such as the presence of other sources of nectar/pollen in the foraging area. In general, a crop can be considered as unattractive to bees when it is harvested before flowering. Some plants that are intrinsically unattractive to bees may be visited by bees because of extra floral nectaries (e.g., in field beans) or honeydew produced by aphids. As a basis for applying the assessment scheme depicted in Figure 10.2, full details of the product and the intended use must be available. (→ 2)

Au: Please check the points given under the headings “Sprayed Treatments” & “Soil or Seed Treatment With Systemic Active Substances” and their cross references in the text for correctness

2a and 2b. Is exposure of adult/immature stages of bees possible?

Based on the information from the product and the intended application it has to be decided whether exposure of adult bees and immature stages (larvae and pupae, brood) can be excluded. The justification has to take into account all routes of exposure that may be relevant to the intended use, for example, through residues on flowers or in flower matrices (e.g., pollen, nectar), and as for non-*Apis* bees in leaves, soil, etc. (Table A6.1).

The screening step has to be initiated if exposure of adult bees (→ 3a) or immature stages (→ 3b) to the active ingredient (a.i.) cannot be excluded. Further risk assessment is not required in cases where exposure can be ruled out for both adults and immature stages of bees (→ 6).

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TABLE A6.1

Likelihood of Exposure to *Apis* and Non-*Apis* Bees from Various Routes

Exposure	<i>Apis</i>		Non- <i>Apis</i>	
	Adults	Larvae	Adults	Larvae
Nectar	+++ ¹	+	+ to +++	+
Pollen	+ to +++	++ ²	+ to +++	++ to +++
Water ^a	+ to ++	+ ³	+	+
Nesting material	+ ⁴	+ ⁴	+ to +++ ^{4,5}	+ to +++ ^{6,7,8}
Exposure to soil	±	—	— to +++	— to +++
Foliar residues				
(Contact and direct spray)	+++	—	+++	— to +++
Direct spray	+++ ⁹	—	+++ ⁹	—

^aCollect water for cooling (evaporative cooling; take up into crop, regurgitate it, and flap wings to distribute) and honey production.

¹Particularly for nurse bees; ²bee bread; ³provided by nurse bees; ⁴wax; ⁵leaves and soil for cement; ⁶leafcutting bees; ⁷soil used to cap cells; ⁸exposure to soil; ⁹at flowering.

3a. Assess the toxicity of active ingredient (a.i.) to *Apis mellifera* adults: Establish acute oral and contact LD50, calculate HQ (appl. rate/LD50). Is HQ below the trigger value (e.g., HQ <50)?

Acute oral and contact toxicity of the active ingredient to adult honey bees should be determined in appropriate laboratory tests generating median lethal doses (LD50) for both routes of exposure (cf. Chapter 7). The highest intended field application rate is used to estimate possible exposure in comparison to the most sensitive of these LD50 endpoints. A hazard quotient (HQ) is calculated by dividing the application rate (g a.i./ha) by the most sensitive acute toxicity endpoint (µg/bee). The resulting HQ does not directly specify the relation of exposure level and toxicity since the numerator (application rate in terms of g a.i./ha) and denominator (LD50 in terms of µg/bee) of the HQ are in different units of measurement. Rather, it is used as a preliminary screen to indicate whether a level of exposure may lead to adverse effects (i.e., that a presumption of minimal risk cannot be made) based on empirical incident data. This initial HQ calculation is used as an indicator of risks in the European regulatory process and has been compared to EU incident data. Comparisons of screening-level HQ values with incident data have indicated that adverse effects in the field are not observed when HQ values are greater than 50 (see Mineau et al., 2008). In this flow chart, the screening-level HQ trigger of 50 is given as an example of value that is used in Europe for screening purposes (EC, 2010); however, regulatory authorities must develop their own triggers for moving to more refined assessments. The intent here is to demonstrate that at a screening level, relatively coarse measures of exposure are used in combination with relatively simple measures of effects to determine whether risk can be presumed low. Where HQ exceeds the trigger value a higher tier risk assessment or consideration of risk mitigation measures is required (→ 7). Otherwise the risk to adult honey bees (*Apis* bees) may be assessed to be low and consideration of possible effects on non-*Apis* bees is the next step of the screening procedure (→ 4a).

3b. Assess the toxicity of a.i. to *A. mellifera* larvae: Establish NOEL, calculate TER, is TER >1?

Chronic toxicity of the active ingredient to honey bee larvae should be determined in an appropriate laboratory test generating a NOEC for the brood development including adult emergence weight (cf. Chapter 8). For

risk assessment, this toxicity endpoint is compared to the exposure of honey bee larvae via contaminated food items. If chemical/crop specific exposure data are not available, then default exposure estimates may be determined through information from residue analysis data (see Chapter 7 for more details). Toxicity and exposure data (expressed in same measurement units of $\mu\text{g/kg}$) are related in a TER calculation ($\text{TER} = \text{NOEC}$ divided by predicted exposure). The resulting TER is compared to an appropriate trigger and any value above that trigger indicates a presumption of minimal risks. In the flow chart, a trigger of 1 is used based on the presumption that maximum residues measured in pollen have not exceeded $100 \mu\text{g/kg}$ and that using a value of $1000 \mu\text{g/kg}$ would likely be considered protective. Again, appropriate exposure values and triggers must be determined by the regulatory authority; however, at this level of refinement, potential risks are determined from toxicity data on bee brood and rely on the no-observed-effect concentration.

4a. Assess possible impacts to non-*Apis* adults using NTA data as surrogate: If HQ for *Apis* is between 5 and 50, consider NTA: Calculate HQ, is HQ <2?

When specific data on the toxicity of the compound to adult non-*Apis* bee species are lacking, potential risk may be estimated from the data available on the honey bee and if available in the dossier, the use of data on other non-target arthropods (NTA). A possible tiered approach using these data, to screen for the need of a risk assessment specific to non-*Apis* bees that would use dedicated data is presented thereafter. Initially the HQ calculated under point 3a using the honey bee LD50 could be used as a trigger of concern for possible effects on non-*Apis* bees. This HQ value would then be compared to a trigger value lower by an order of magnitude to account for interspecies variability of toxicity data. Thus the HQ calculated under point 3a shall be lower than five for acceptable risks to be concluded for adult honey bees and adult non-*Apis*. The order-of-magnitude increase in the trigger is intended to account for interspecies variability. In the case of $5 < \text{HQ} < 50$, data on NTA species would be considered in order to conclude about the level of concern of the product for non-*Apis* bees, taking into consideration the level of risk for NTA species and how representative the test species are of non-*Apis* bees expected to frequent the crop, etc. As an example, in the risk assessment scheme for NTA performed in the EU, the laboratory toxicity endpoint for the most sensitive NTA species is compared to the maximum application rate in an HQ calculation (where the toxicity endpoint is also expressed as a rate (g a.i./ha)) (Candolfi et al., 2001). This HQ is assessed against a trigger value of 2. Where the HQ value for NTA exceeds this trigger value, it is concluded that risk to non-*Apis* cannot be excluded and that risk estimates should be further refined. This refinement could consider the generation of specific adult toxicity data with a non-*Apis* species before a higher tier risk assessment or consideration of risk mitigation measures (\rightarrow 5a). If mitigation measures are considered, then the effect of these measures on potential exposure should be considered using the same process as just described from the point where potential risk could not be presumed low/minimal.

If this HQ for NTA is below the trigger value, the risk to adult non-*Apis* bees may be considered minimal (\rightarrow 6).

5a. Establish adult oral and contact LD50 for a non-*Apis* bee species: Calculate HQ, is HQ <50?

The screening step 3a may be repeated using specific toxicity data generated in tests with a non-*Apis* bee species. For further details on laboratory studies on non-*Apis* bees, see Chapter 8. Where the HQ exceeds the trigger value of 50, a higher tier risk assessment or consideration of risk mitigation measures is required (\rightarrow 7). For HQ values below the trigger, risk to larvae of non-*Apis* bees is considered minimal (\rightarrow 6).

5b. Establish larval NOEL for relevant Non-*Apis* bee species: Calculate TER, is TER >10?

The screening step 3b may be repeated using specific toxicity data generated in tests with a non-*Apis* bee species. For further details on laboratory studies on non-*Apis* bees, see Chapter 8. Where TER is below the

trigger value of 10, a higher tier risk assessment or consideration of risk mitigation measures is required (→ 7). For TER values above 10, the risk to larvae of non-*Apis* bees is considered minimal (→ 6).

6. Presumption of minimal risk

If exposure can be excluded or the criteria in the screening step are met for both adult bees and larvae, then a minimal risk to honey bees and/or non-*Apis* bees can be presumed. A minimal risk for honey bees and/or non-*Apis* bees can also be presumed if treatments in higher tier semi-field and field tests result in no significant difference compared to the untreated control when evaluated against the protection goals. Further risk mitigation measures are not required.

7. Continue with higher tier risk assessment or consider risk mitigation measures and reassess

If in the screening step the criteria for adult bees or larvae are not met, a higher tier risk assessment (depicted in Figures A6.1 and A6.2) should be performed (→ 8). The screening step may be repeated to consider specific risk mitigation measures that exclude or mitigate exposure (e.g., by reducing the application rate, avoiding the exposure to residues during flowering) (→ 2). For further considerations of risk mitigation measures, see Chapter 11.

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8. Is higher tier risk assessment triggered by failing the screening step with non-*Apis* bees?

Concerns identified in the screening procedures and which are not addressed through mitigation, may then be further refined through semi-field or field tests (→ 9). If in the screening steps potential risks were identified for non-*Apis* species (adults or larvae) that will be further refined in a higher tier study, then the assessor should consider whether a higher tier study with honey bees would also be representative of the concerns identified for non-*Apis* bees in the screening step (→ 13).

9. Is higher tier risk assessment triggered by failing the screening step with regard to honey bees?

If in the screening step the criteria for *Apis* (adult bees or larvae) are not met, a semi-field or field test should be performed to further refine potential concerns. (→ 10 or 11). In transitioning from the use of laboratory-based studies on individual bees to semi-field and field toxicity studies typically conducted at the colony level, test conditions are intended to reflect more realistic exposure conditions. Unlike the lower tier studies, though exposure is incorporated into the results of the semi-field and field studies such that the question being asked is whether there is an adverse effect under the conditions tested. As measurement endpoints are selected in higher tier studies to directly reflect assessment endpoints that are in turn intended to address protection goals, these studies simply answer a yes/no question and do not require risk estimates, that is, no HQ or TER is calculated.

10 and 11. Assess the effects of the a.i. to *A. mellifera* in a semi-field or a field test: Do results indicate minimal risk (no significant difference to control)?

Concerns raised in the screening procedure may be investigated through a semi-field test where possible effects are assessed against the criteria related to the protection goals. This is to say that measurement endpoints should be readily related to assessment endpoints which in turn reflect protection goals. For example, if a protection goal is to ensure pollination services, then having sufficient forage strength in a colony is important. Therefore, adult and larval bee survival is a reasonable assessment endpoint and the number of dead bees in traps and/or brood termination rates may be reasonable measurement endpoints to reflect that assessment endpoint.

The choice between semi-field and field testing depends on the profile of the product as, for example, the expected duration of exposure, the possibility of occurrence of effects, the nature of the anticipated effects.

Appendix 6: Detailed Description of the Proposed Overall Risk Assessment Scheme

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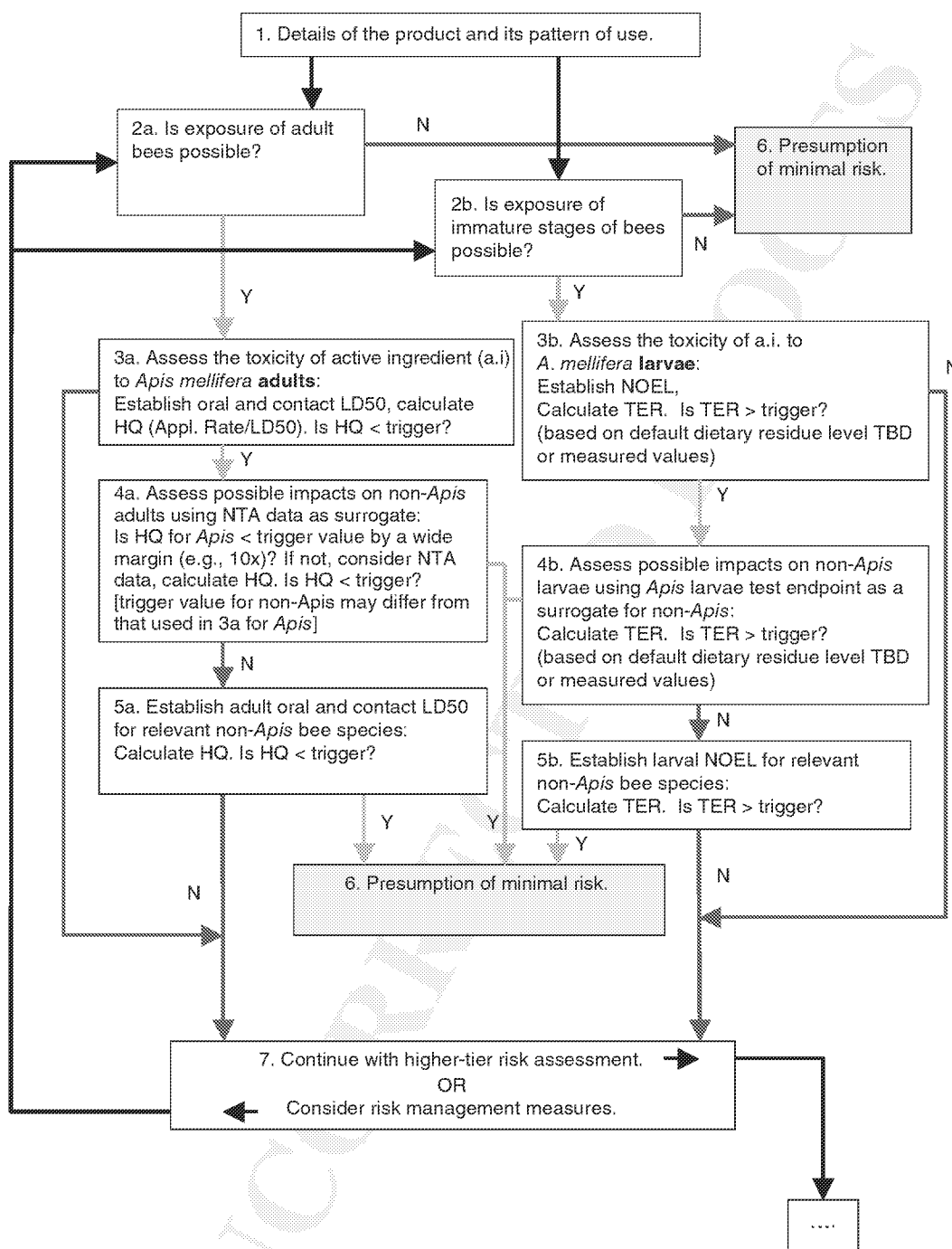


FIGURE A6.1 Insect pollinator screening-level risk assessment process for foliarly applied pesticides. (For a color version, see the color plate section.)

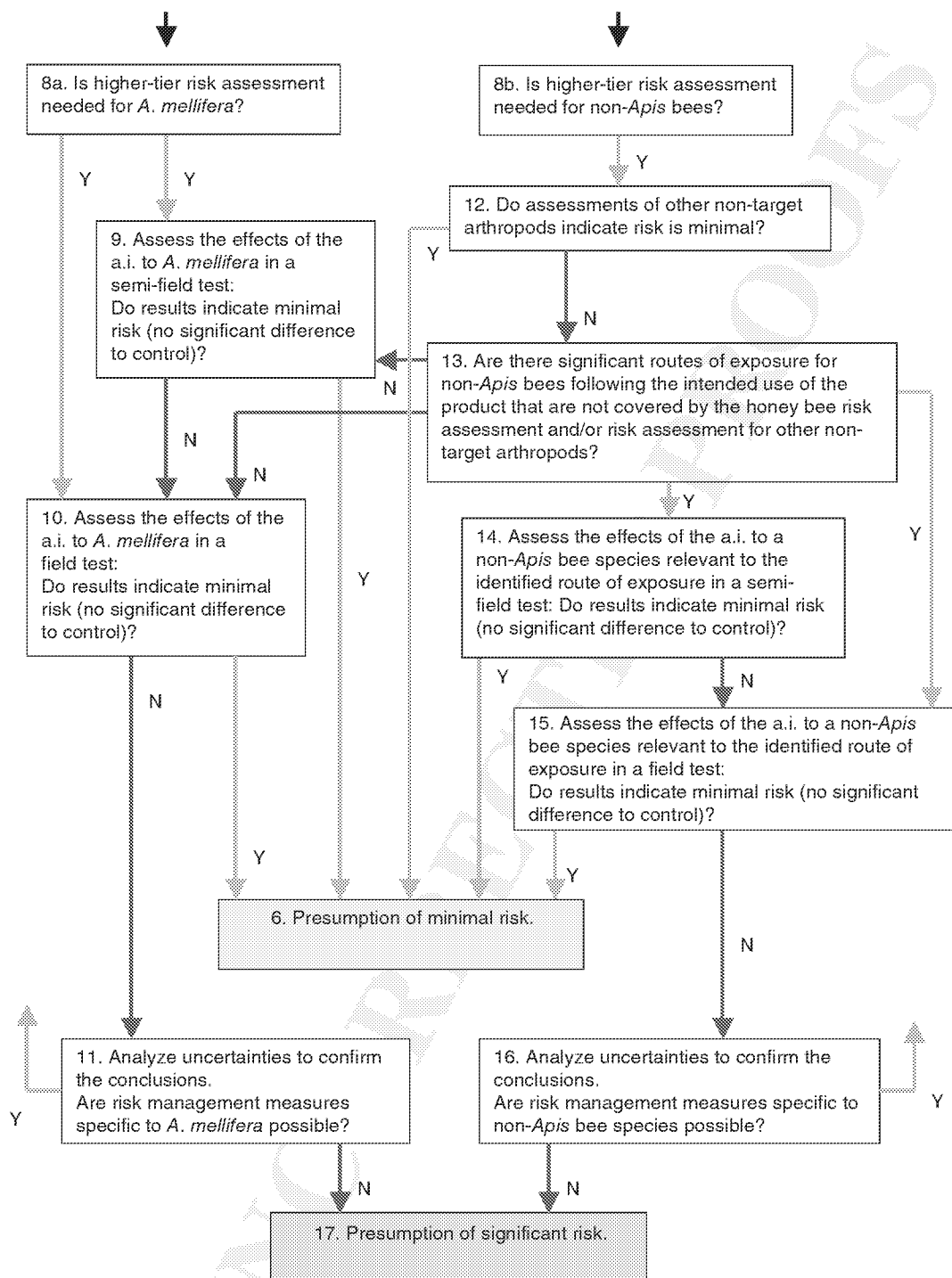


FIGURE A6.2 Higher tier (refined) risk assessment process for foliarly applied pesticides. (For a color version, see the color plate section.)

This choice is a case-by-case decision, but the design of semi-field and field studies should be informed by the information deduced from lower tier testing and other relevant lines of evidence, for example, incident data.

Semi-field testing (cage, tunnel, or tent tests) is a suitable option before full field testing. The advantage of semi-field testing is that mortality is easier to assess and exposure of bees to the test compound is more readily ensured since bees are confined within a tent and cannot forage elsewhere. In addition, if an accurate quantification of exposure is needed, semi-field studies may provide more reproducible residue levels due to the relative protection from weather conditions.

Semi-field as well as full field tests aim at evaluating the level of effects that may be expected on bees exposed to the product under realistic conditions, that is, through the crop having been treated at proposed application rates. Because the conditions of exposure of bees are more reflective of actual use conditions, the results of these trials may be directly used in risk assessment (see Chapter 9).

The design of semi-field/field testing may also follow a tiered approach. In the first instance, semi-field tests should be designed in order to maximize the exposure of bees to residues resulting from an application. For sprayed products, the demonstration of acceptable effects in a semi-field or field test performed on a "standard crop" (e.g., wheat) made artificially attractive through a sugar solution and treated at the maximum application rate at flowering may be considered as protective for any crop that may be further treated with the product. Further steps may consider bee-attractive crops treated at flowering (e.g., phacelia), and then the specific crops on which the compound will actually be applied as a highest tier when a treatment at flowering cannot be excluded. Further on, the possibility of an exposure outside the flowering period of the crop through, for example, spray drift onto flowers in vegetated areas or onto flowering weeds within the crop (e.g., understory of orchards), should also be considered in the trials, if triggered by the lower tiers. In the case of soil/seed treatments, it may be more difficult to identify a surrogate (worst case) crop as the exposure results from systemic properties and the attractiveness of the crop to bees. For both sprayed and soil/seed treatments, in the case of systemic activity, if the substance or its residues are persistent and the product may be used on several crops in a rotation, the potential accumulation in soil and subsequent effect on in-plant residues should be considered in the study protocol.

For both semi-field and field trials, it should be demonstrated that the test bees were actually exposed under the environmental conditions (especially weather conditions in case of field trials) of the study. The use of a toxic standard (semi-field trials) or pollen collection and residue analysis, may also help to document exposure. A quantified assessment of the exposure is particularly important for systemic products, as reference substances for systemic products are difficult to define since they too would be dependent on crop properties. There should always be a comparable untreated control in order to provide a reference point against which to compare the test treatments. While positive controls (toxic reference chemicals) are frequently used in laboratory and semi-field studies, they are not typically used in full-field studies. Therefore, it is not possible to demonstrate definitively that the study design is sufficient to detect treatment effects and it is important to document exposure through residue analyses.

For honey bees, suitable methods for semi-field and field trials are discussed in OEPP/EPPO (2010) (see Chapter 9) which have been defined for sprayed treatment and can be adapted to soil/seed treatments (systemic activity). These studies may also be modified for specific assessments with honey bees, for example, repellency and other behavioral effects, effects of aged residues or for specific testing of brood effects. Possible adaptations for non-*Apis* species are discussed in Chapter 9.

The interpretation of semi- and full-field study results is further detailed in Chapter 9. It should rely on a comparison of effects in the test chemical treatments and in the concurrent negative control. If the semi-field test treatment results in no significant difference from untreated controls in lethal and sub-lethal effects (i.e., survival, growth, reproduction, and foraging behavior), a minimal risk is indicated (→ 6). Otherwise a higher tier evaluation using a field test has to be performed (→ 11).

12. Are risk mitigation measures specific to *A. mellifera* possible?

If the results of higher tier semi-field and field tests indicate that the protection goals are not met, the assessment scheme may be reiterated considering specific risk mitigation measures mitigating the exposure of honey bees (→ 2). Note in this respect that semi-field and field tests may be appropriately adapted in order to check for the efficiency of risk mitigation measures to reduce exposure to and subsequent impact from treatment residues on bees.

13. Are there significant routes of exposure for non-*Apis* bees that are not covered by the honey bee risk assessment and/or risk assessment for other NTA?

In any case when a risk assessment for non-*Apis* bees is triggered and a refined risk assessment is available for honey bees and NTAs, it may be interesting to discuss the extent these risk assessments address as part of the risk issues relative to non-*Apis* species. As an example, concerns with effects on non-*Apis* bees identified at the lower levels may in some cases be addressed by semi-field or field tests with honey bees as for example, where no additional significant routes of exposure for non-*Apis* bees have to be taken into account. Furthermore, higher tier field data generated with NTA species may also address these concerns provided the routes of exposure are comparable to those for non-*Apis* bees (Table 10.3, see Chapter 9). If these data are considered suitable surrogates and if the examination of these data results in no significant risk with regard to the protection goals, then a minimal risk to non-*Apis* bees is indicated (→ 6). Otherwise semi-field or field tests with non-*Apis* bees should be considered to address the concern (→ 14).

14 and 15. Assess the effects of the a.i. to a non-*Apis* bee species relevant to the identified route of exposure in a semi-field or a field test: Do results indicate minimal risk (no significant difference to control)?

Potential risks identified in the screening-level assessment may be addressed by appropriately designed semi-field tests where possible effects are assessed against the evaluation criteria related to the protection goals. The derivation of evaluation criteria for specific protection goals is discussed in Chapter 4. For further details on semi-field studies on non-*Apis* bees, see Chapter 9. As previously developed in the case of honey bees, the choice between a semi-field test or a full-field study depends on the outcome of lower tier studies and should also consider choices made for honey bees. If the results of semi-field or field test, in conjunction with information from lower tier studies and other relevant data indicate no significant difference in relevant lethal and sub-lethal effects compared to untreated controls, minimal risk is indicated (→ 6).

Otherwise, further risk mitigation may be considered or the risk has to be presumed as significant (→ 16).

16. Are risk mitigation measures specific to non-*Apis* bee species possible?

If the results of higher tier semi-field and field tests on non-*Apis* indicate that the protection goals are not met, the assessment scheme may be reiterated considering specific measures designed to mitigating the exposure of non-*Apis* bees (→ 2).

Note in this respect that semi-field and field tests may be appropriately adapted in order to check for the efficiency of risk mitigation measures to limit the exposure and potential impact of treatment residues on non-*Apis* bees.

17. Presumption of significant risk

If there are no measures available to sufficiently mitigate the risk to honey bees and/or non-*Apis* bees indicated by the evaluation of the results of higher tier semi-field and field tests against the protection goals, then a significant risk has to be presumed.

A6.2 SOIL OR SEED TREATMENT WITH SYSTEMIC ACTIVE SUBSTANCES

1. Details of the product and its pattern of use

As a basis for applying the assessment scheme, details of the product and the intended use must be available, especially the crop, the formulation type, type and timing of application, as well as the application rate (g a.i./ha). In addition it has to be determined whether the active ingredient has systemic properties, that is, significant portions of the compound are translocated in the plant resulting in residues of concern in plant matrices like nectar, pollen, and leaves that might lead to exposure of bees (→ 2). If persistent soil residues may give rise to uptake of the substance by succeeding (rotational) crops, the same considerations with regard to attractiveness of these crops to bees apply as discussed in the description of the risk assessment scheme for spray applications. Restrictions concerning the choice of succeeding crops may be considered as risk mitigation measures.

2a and 2b. Is exposure of adult/immature stages of bees possible?

Based on the information on the product and its intended application it has to be decided whether exposure of adult bees and immature stages (larvae and pupae, brood) can be excluded. The justification has to take into account all routes of exposure that may be relevant to the intended use, for example, through residues on flowers or in flower matrices (e.g., pollen, nectar), and as for non-*Apis* bees in leaves, soil, etc. (Table 10.3).

The screening step should be initiated if exposure of adult bees (→ 3a) or immature stages (→ 3b) to the active ingredient cannot be excluded. Further risk assessment is not required in cases where exposure can be ruled out for both adults and immature stages of bees (→ 6). Special routes of exposure of bees as a result of soil or seed treatment application of active substances with systemic properties may not be covered by the risk assessment scheme for spray application. The exposure of bees to residues of a systemic product may occur through transfer of residues taken up by the roots from the seed coating or soil and distributed to the upper (apical) parts of the plant and in particular in matrices of interest to bees (pollen, nectar, and honeydew) if the crop is visited by bees. The resulting residue of concern may comprise the active substance and/or systemic soil degradation products or metabolites formed in the plants. Honeydew might not be considered a relevant route because the concentration of a systemic compound translocated in the phloem and reaching honeydew without harming aphids should in principle not be capable of harming bees foraging on the honeydew, unless the compound is highly selective toward non-aphid insects. If there is uncertainty regarding potential residues in honeydew because there is insufficient information on selectivity available in the registration dossier, a dedicated evaluation according to the present risk assessment scheme would be triggered. Information derived from residue studies and plant metabolism studies is in general sufficient to identify if the substance is internally distributed within the plant during its growth, and if it is further degraded into major degradation products. Similarly, possible uptake and distribution in plants of major soil degradation products could be identified in these residue studies as well. The sensitivity (i.e., limits of quantification and detection) of the analytical methods that are used in the residue studies must be checked in order to ensure that they are low enough to detect residue levels that exert toxic effects to bees. If it is uncertain whether the detection methods are sufficiently sensitive, additional investigations have to be considered to demonstrate the absence of residue translocation at potentially toxic levels. Studies that specifically investigate the presence of residues in flowers, nectar, or pollen may be considered as an option for the generation of data to refine the predicted exposure of bees.

Other routes of exposure as a consequence of soil or seed treatment application (e.g., drift of abraded treated seed coating dust into adjacent areas attractive to bees) are not specific to systemic active substances and therefore not addressed in this risk assessment scheme. It should be noted that the emission and dispersion of dusts at sowing is considered as reflecting a poor quality sowing and/or formulation practices that could

be mitigated to reduce potential exposure to a minimum level. Therefore measures aiming at reducing the emission and dispersion of dusts at sowing should be considered.

3a. Assess the toxicity of a.i. to *A. mellifera* adults (oral exposure): Establish Oral LD50, calculate TER, compare TER to an appropriate trigger value

The acute oral toxicity of the active ingredient to adult honey bees should be determined in appropriate laboratory tests generating median lethal doses (LD50) (Chapter 8). The highest intended field application rate is used to estimate possible exposure in comparison to the most sensitive of acute contact and acute oral LD50 endpoints.

For risk assessment, the LD50 is set into relation to the exposure of adult honey bees. For this purpose, a default dietary residue level may be used, as for example the value of 1 mg a.i./kg proposed by the EPPO (2010). Measured residue levels may also be used as a refinement of exposure estimates. As exposure estimates should reflect the maximum expected residue levels for a “worst-case” assessment, the measured residue in plant matrices to be used as a refinement of exposure estimates for TER calculation could, for example, be based on the upper 90th percentile of residue data for the relevant crop for comparison to the most sensitive acute LD50. Toxicity and exposure data expressed in same units are related in a TER calculation ($TER = LD50 \text{ divided by predicted exposure}$) where residue concentrations have to be expressed in terms of daily uptake per bee ($\mu\text{g/kg}$). The calculated TER is assessed against an appropriate trigger value. A trigger value of 10 may, for example, be applied indicating that the predicted exposure is lower than the acute toxicity by at least one order of magnitude and the margin of safety achieved can be regarded as sufficient to cover the uncertainty related to longer exposure periods and possible related increased sensitivity (EPPO, 2010).

Where the TER is below the trigger value, a higher tier risk assessment or consideration of risk mitigation measures is required (\rightarrow 8). As a refinement option a prolonged toxicity test in the laboratory may be considered (\rightarrow 4a). Otherwise, the risk to adult honey bees is assessed to be low and an evaluation of possible effects on non-*Apis* bees is the next step of the screening procedure (\rightarrow 5a).

3b. Assess the toxicity of a.i. to *A. mellifera* larvae: Establish NOEL, calculate TER, compare TER to an appropriate trigger value

Chronic toxicity of the active ingredient to honey bee larvae should be determined in an appropriate laboratory test generating an NOEC for the brood development including adult emergence weight (Chapter 8). For risk assessment, this toxicity endpoint is compared to the exposure of honey bee larvae via contaminated food items. If chemical/crop-specific exposure data are not available, then default exposure estimates may be determined through information from residue analysis data (see Chapter 7 for more details). Toxicity and exposure data (expressed in same measurement units of $\mu\text{g/kg}$) are related in a TER calculation ($TER = NOEC \text{ divided by predicted exposure}$). The resulting TER is compared to an appropriate trigger and any value above that trigger indicates a presumption of minimal risks. In the flow chart, a trigger of one is used based on the presumption that maximum residues measured in pollen do not exceed 100 $\mu\text{g/kg}$ and that using a value of 1000 $\mu\text{g/kg}$ would likely be considered protective. Again, appropriate exposure values and triggers must be determined by the regulatory authority; however, at this level of refinement, potential risks are determined from toxicity data on bee brood and rely on the NOEC.

4a. Assess the oral toxicity of a.i. to *A. mellifera* Adults in a prolonged (10 day) test: Establish oral NOEL, calculate TER, and compare TER to an appropriate trigger value

As a refinement option the NOEL derived from a 10-day toxicity test with oral exposure may be taken into account before embarking on a higher tier risk assessment. The NOEL is related to the potential exposure of adult honey bees via consumption of contaminated food items (default value as for example 1 mg a.i./kg

or measured residue data). A TER value is calculated by dividing NOEL by predicted exposure expressed in the same units of measurement. In this case, as the effects are monitored over a 10-day period, the average (or time-weighted average) of residue levels is a more appropriate exposure estimate in a TER calculation. The calculated TER is assessed against an appropriate trigger value. A trigger value of one may be applied as the toxicity endpoint is related to the NOEL. Where the TER is below the trigger value, a higher tier risk assessment or consideration of risk mitigation measures is required (→ 8). Otherwise the risk to adult honey bees is assessed to be low and consideration of possible effects on non-*Apis* bees is the next step of the screening procedure (→ 5a).

5a. Assess possible impacts on non-*Apis* adults using NTA Data as surrogate: If TER for *Apis* is Between 10 and 100, consider NTA data

When specific data on the toxicity of the compound to adult non-*Apis* bee species are lacking, potential risk may be estimated from the data available on the honey bee and if available in the dossier, the use of data on other NTA.

Explore the NTA data package to ascertain whether there is likely to be a significant risk to non-*Apis* bees by considering the characteristics of each species tested, for example, *Aleochara bilineata* may give some evidence concerning soil-dwelling species and *Aphidius* sp. on nectar-feeding species. Where a risk to non-*Apis* bees (as estimated using NTA) cannot be excluded, more refinement is considered necessary. This refinement could consider the generation of specific adult toxicity data with a non-*Apis* species before a higher tier risk assessment or consideration of risk mitigation measures (→ 6a). If mitigation measures are considered, then the effect of these measures on potential exposure should be considered using the same process as just described from the point where potential risk could not be presumed low/minimal. If the risk to NTA is considered to be minimal, the risk to adult non-*Apis* bees may be considered minimal (→ 7).

6a. Establish adult oral LD50 for a non-*Apis* bee species: Calculate TER, compare TER to an appropriate trigger value

The screening step 3a may be repeated using specific toxicity data generated in tests with a non-*Apis* bee species. For further details on laboratory studies on non-*Apis* bees, see Chapter 8. For risk assessment, the LD50 endpoint is set into relation to the exposure of adult non-*Apis* bees. For this purpose a worst-case default dietary residue level of 1 mg a.i./kg (EPPO 2010) or measured residue data in relevant food items may be used. Toxicity and exposure data expressed in the same units are expressed as a ratio in a TER calculation ($\text{TER} = \text{LD50} \text{ divided by predicted exposure}$) where residue concentrations have to be expressed in similar terms, that is, daily uptake per bee. The calculated TER is assessed against an appropriate trigger value. A trigger value of 10 indicating that the predicted exposure is lower than the acute toxicity by at least one order of magnitude may be considered to be appropriate also for non-*Apis* bees. Where TER is lower than the trigger value, a higher tier risk assessment or consideration of risk mitigation measures is required (→ 8). Otherwise the risk to adults of non-*Apis* bees is considered minimal (→ 7).

4b. Assess possible impacts on non-*Apis* immature stages: If TER for *Apis* is between 1 and 10, establish larval NOEL for relevant non-*Apis* bee species (→ 5b). Otherwise the risk to immature non-*Apis* bees is considered minimal (→ 7)

Lacking specific data on the toxicity of the compound on immature stages of non-*Apis* bee species, the assessment of possible effects on this group in the screening procedure can utilize data on honey bees. As a trigger of concern for possible effects on non-*Apis* bees the TER calculated under point 3b using a honey bee larval NOEC is compared to a value higher by an order of magnitude to account for interspecies variability of toxicity data. Where this TER is below a trigger value of 10 a refinement of the screening step may be

considered generating specific toxicity data with immature stages of non-*Apis* bee species before a higher tier risk assessment or consideration of risk mitigation measures is required.

5b. Establish larval NOEL for a non-*Apis* bee species: Calculate TER, compare TER to an appropriate trigger value

The screening step **3b** may be repeated using specific toxicity data generated in tests with a non-*Apis* bee species. For further details on laboratory studies on immature stages of non-*Apis* bees, see Chapter 8. Toxicity and exposure data expressed in the same units are expressed as a ratio in a TER calculation ($TER = NOEC$ divided by predicted exposure concentration). The calculated TER is assessed against an appropriate trigger value. A trigger value of 10 indicating that the predicted exposure is lower than the acute toxicity by at least one order of magnitude may be considered to be appropriate also for non-*Apis* bees. Where TER is below the trigger value, a higher tier risk assessment or consideration of risk mitigation measures is required (\rightarrow 8). For TER values that are higher than the trigger, the risk to larvae of non-*Apis* bees is considered minimal (\rightarrow 7).

7. Presumption of minimal risk

If exposure can be excluded or the assessment criteria in the screening step are met for both adult bees and larvae a minimal risk to honey bees and non-*Apis* bees can be presumed.

A minimal risk for honey bees and non-*Apis* bees can also be presumed if treatments in appropriate higher tier semi-field and field tests result in no significant difference compared to the untreated control when evaluated against the protection goals. Further risk mitigation measures are not required.

8. Continue with higher tier risk assessment or consider risk mitigation measures and reassess

If in the screening step the assessment criteria for adult bees or larvae are not met, a higher tier risk assessment should be performed (\rightarrow 9). Alternatively the screening step may be repeated considering specific risk measures excluding or mitigating exposure (\rightarrow 2). For further considerations on risk mitigation measures, see Chapter 12.

9. Is higher tier risk assessment triggered by failing the screening step with regard to non-*Apis* bees?

Concerns identified in the screening procedure have to be addressed in semi-field or field tests with honey bees (\rightarrow 10). If in the screening step the criteria for adult bees or larvae are not met with regard to non-*Apis* bees, it must be determined whether a higher tier study with honey bees are sufficient to cover concerns identified for non-*Apis* bees in the screening step (\rightarrow 14).

10. Is higher tier risk assessment triggered by failing the screening step with regard to *A. mellifera*?

If in the screening step the criteria for adult bees or larvae are not met only with respect to honey bees, a semi-field or field test should be performed to address the concern (\rightarrow 11 or 12). (Note that the higher tier part of the risk assessment schemes is identical for both spray and soil/seed treatment application. Due to an additional step in the screening procedure, the numbering of the steps in the higher tier risk assessment scheme for soil/seed treatment application is different (+ 1).)

11. and 12. Assess the effects of the a.i. to *A. mellifera* in a semi-field or a field test: Do results indicate minimal risk (no significant difference to control)?

See 10 and 11 in the risk assessment flowchart for sprayed treatments.

Where in the semi-field test or in the field test treatment results in no significant difference in lethal and sub-lethal effects on survival, growth, reproduction, and foraging behavior compared to untreated

control, a minimal risk is indicated (→ 7). Otherwise a higher tier evaluation a field test has to be performed (→ 12).

13. Risk mitigation measures specific to *A. mellifera* possible?

Where the results of higher tier semi-field and field tests indicate that the protection goals are not met, the assessment scheme may be reiterated considering specific measures to mitigate the exposure of honey bees (→ 2). Note in this respect that semi-field and field test may be appropriately adapted in order to check for the efficacy of risk mitigation measures to limit the exposure and subsequent impact on bees.

14. Are there significant routes of exposure for non-*Apis* bees that are not covered by the honey bee risk assessment and/or risk assessment for other NTA?

In any case when a risk assessment for non-*Apis* bees is triggered and a refined risk assessment is available for honey bees and NTAs, it may be interesting to discuss the extent to which these risk assessments address part of the risk issues relative to non-*Apis* species. As an example, concerns with effects on non-*Apis* bees identified at the lower levels may in some cases be addressed by semi-field or field tests with honey bees, as for example, where no additional significant routes of exposure for non-*Apis* bees have to be taken into account. Furthermore, higher tier field data generated with NTA species may also address these concerns provided the routes of exposure are comparable to those for non-*Apis* bees (Table 10.3, see Chapter 9). If these data can serve as surrogates and if the examination of these data results in no significant risk with regard to the protection goals, then a minimal risk to non-*Apis* bees is indicated (→ 7). Otherwise semi-field or field tests with non-*Apis* bees have to be performed to address the concern (→ 15 or 16).

Au: Please check and confirm whether “(Table 10.3, see Chapter 9)” has been given as intended in the sentence “Furthermore, higher tier field data [...] non-*Apis* bees (Table 10.3, see Chapter 9).”

15. Assess the effects of the a.i. to a non-*Apis* bee species relevant to the identified route of exposure in a semi-field test: Do results indicate minimal risk (no significant difference to control)?

Concerns raised in the screening procedure may be addressed by appropriately designed semi-field/field tests where possible effects are assessed against the criteria intended to reflect the protection goals. The derivation of assessment criteria for specific protection goals is discussed in Chapter 4. For further details on semi-field studies on non-*Apis* bees, see Chapter 9. Where in the semi-field test treatment results in no significant difference in relevant lethal and sub-lethal effects compared to untreated control, a minimal risk is indicated (→ 7). Otherwise in a higher tier evaluation a field test should be performed (→ 16).

16. Assess the effects of the a.i. to a non-*Apis* bee species relevant to the identified route of exposure in a semi-field or a field test: Do results indicate minimal risk (no significant difference to control)?

Concerns raised in the screening-level assessment may be addressed by appropriately designed semi-field tests where possible effects are assessed against the evaluation criteria related to reflect the protection goals. The derivation of evaluation criteria for specific protection goals is discussed in Chapter 4. For further details on semi-field studies on non-*Apis* bees, see Chapter 9. As for honey bees, the choice between a semi-field test and a full-field study depends on the outcome of lower tier studies and should also consider decisions for honey bees. If the results of semi-field or field test, in conjunction with information from lower tier studies and other relevant data indicate no significant difference in relevant lethal and sub-lethal effects compared to untreated controls, minimal risk is indicated (→ 7). Otherwise, further risk mitigation may be considered or the risk has to be presumed as significant (→ 17).

17. Risk mitigation measures specific to non-*Apis* bee species possible?

Where the results of higher tier semi-field and field tests on non-*Apis* bees indicate that the protection goals are not met, the assessment scheme may be reiterated considering specific measures designed to mitigating the exposure of non-*Apis* bees (→ 2).

Note in this respect that semi-field and field test may be adapted in order to determine whether risk mitigation measures actually limit the exposure and potential impact on *non-Apis* bees.

18. Presumption of significant risk

If there are no measures available to mitigate the risk to honey bees and/or *non-Apis* bees indicated by the evaluation of the results of higher tier semi-field and field tests against the protection goals, then a significant risk has to be presumed.

REFERENCES

- Au: Please provide citation in the text for the reference "Alix & Lewis, 2010"**
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